THE DECREASE OF PERMEABILITY PRODUCED BY ANESTHETICS

W. J. V. OSTERHOUT

(WITH SIX FIGURES)

A number of writers hold the view that anesthetics increase permeability, while others believe that anesthetics bring about a decrease of permeability.[1] It appears that the removal of this uncertainty is a necessary step toward a satisfactory theory of anesthesia.

It occurred to the writer that the cause of the discrepancy might lie in the fact that anesthetics produce both an increase and a decrease of permeability, and that quantitative methods might clear up the confusion. An investigation showed that this was the case. It also demonstrated that the characteristic effect of anesthetics is a decrease in permeability.

The investigations were begun in 1912. A brief announcement of some of the principal results has already appeared.[2] The present paper gives the details of these and of later researches and contains additional facts of importance.

Since ether, chloroform, and alcohol deteriorate on standing, especially when in contact with metal or with cork stoppers, special care was taken to obtain pure reagents.[3] Those used were Kahlbaum's or Squibb's.

The experiments were made on tissues of the marine alga *Laminaria saccharina*. The permeability was measured by determining the electrical resistance of the tissues by a method which had been previously described.[4] The method may be illustrated by describing a typical experiment. A lot of tissue which had a net

[1] Cf. HÖBER, Physikalische Chemie der Zelle und der Gewebe, Vierte Auflage. 1914. pp. 466, 597; LILLIE, Amer. Jour. Physiol. **29**:372. 1912; **30**:1. 1912; **31**:255. 1913; Science N.S. **37**:959. 1913; LEPESCHKIN, Ber. Deutsch. Bot. Gesells. **29**:349. 1911; RUHLAND, Jahrb. Wiss. Bot. **51**:376. 1912.

[2] Science N.S. **37**:111. 1913.

[3] Cf. BASKERVILLE, C., Science N.S. **34**:161. 1911.

[4] Science N.S. **35**:112. 1912.

resistance[5] of 750 ohms in sea water was transferred to a mixture consisting of 990cc. sea water+10cc. ether+5cc. sea water

TABLE I

ELECTRICAL RESISTANCE OF *Laminaria saccharina*

Time in minutes	In sea water containing 0.099 M ether (solution not renewed)	In sea water
0	750	750
10	850	....
20	870	....
40	770	750
60	750	....
80	740	....
200	730	....
480	720	740
1500	690	690

All readings were taken at 18° C. or corrected to this temperature.

concentrated by evaporation until its conductivity was about double that of ordinary sea water. This mixture contained approximately 1 per cent by volume of ether (=0.099 M) and had the same conductivity as ordinary sea water. In 10 minutes the resistance had risen to 850 ohms; in 10 minutes more it had fallen to 870 ohms. It continued to fall until it reached 740 ohms, after which it fell very slowly, at about the same rate as the control. The fact that it fell 10 ohms below the starting point is not necessarily to be attributed to any injury, but rather to the fact that the evaporation of the ether increases the conductivity of the sea water which is contained in the apparatus, and in the interstices between the protoplasmic masses. The results are shown in table I and fig. 1.

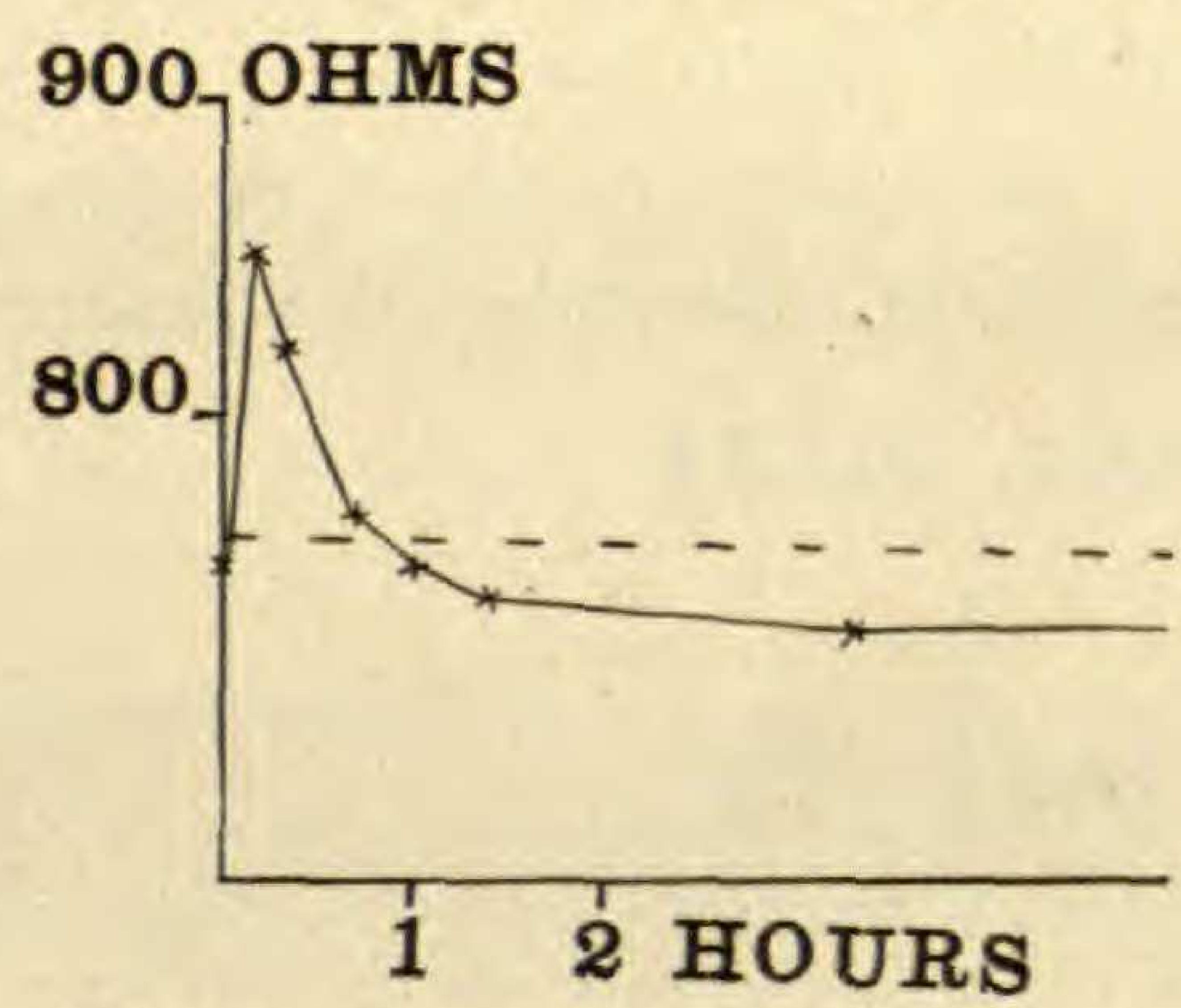

FIG. 1.—Curve showing decrease in permeability (increase in electrical resistance) of *Laminaria saccharina* under the influence of ether (0.099 M in sea water = 1 per cent by volume): the solution was not renewed (the effect of renewing the solution is seen in fig. 2); the dotted line shows the resistance of a control in sea water.

[5] The new resistance is the total resistance minus the resistance of the apparatus; it is therefore the resistance of the tissue taken by itself. The resistances given in this paper are all net resistances.

The permeability may be regarded as equal to the conductivity, or, for convenience, as equal to the conductance. Hence the permeability at the start was 1÷750=0.001333. After treatment

TABLE II

ELECTRICAL RESISTANCE OF *Laminaria saccharina*

Time in minutes	In sea water containing 0.099 M ether (solution renewed every 5 minutes)	In sea water
0	740	850
10	850	....
20	850	....
40	850	850
60	850	....
80	840	850
140	830	850
200	820	850

All readings were taken at 18° C. or corrected to this temperature.

with ether it was 1÷870=0.001149. The decrease in permeability was 0.001333−0.001149=0.000184, or 13 per cent.

In order to see how the evaporation of the ether from the solution influenced the result, another experiment was performed in which the solution was renewed every 5 minutes during the first 60 minutes, and thereafter every 15 minutes. In this way the concentration of ether was kept more nearly constant. It was then found that the resistance rose as before, but did not fall during the first 80 minutes, and after this fell very slowly, so that after 300 minutes it was still 80 ohms above that of the control. At this point the experiment was discontinued. The results are shown in table II and fig. 2.

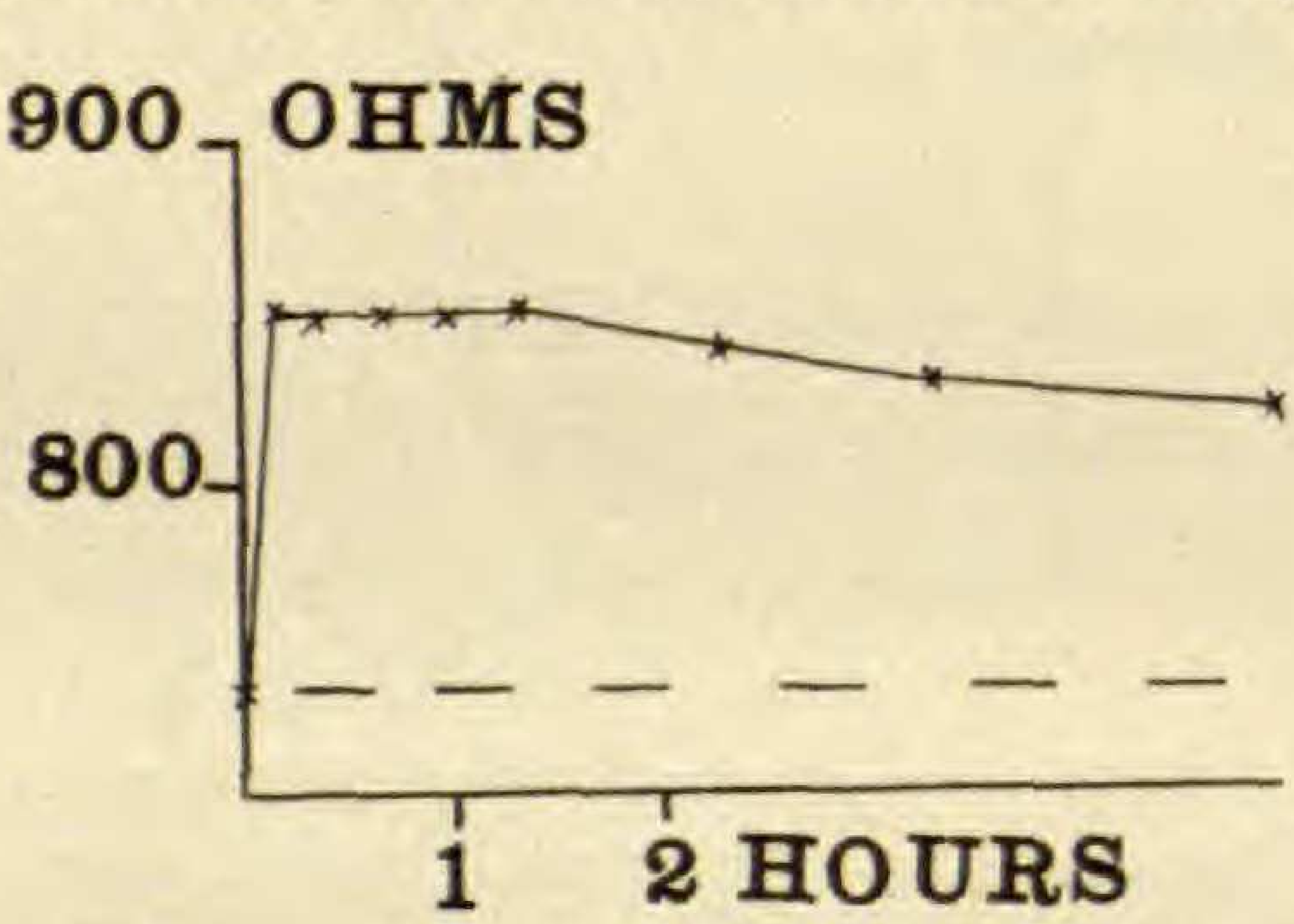

FIG. 2.—Curve showing the decrease in permeability (increase in electrical resistance) of *Laminaria saccharina* under the influence of ether (0.099 M in sea water=1 per cent by volume): the solution was renewed every 5 minutes; the dotted line shows the resistance of a control in sea water.

In order to see whether the effect of the anesthetic could be quickly reversed, some tissue was kept in sea water containing 0.099 M ether for 50 minutes (the solution being renewed every

5 minutes). During this time the resistance rose from 800 to 910 ohms. It was then placed in sea water. At the end of 10 minutes resistance had fallen to 800 ohms. It was left in sea water for 110 minutes and again placed in sea water containing 0.099 M ether (the solution being renewed every 15 minutes). The resistance promptly rose to 910 ohms and remained there for an hour; 240 minutes later, when the experiment was discontinued, the resistance was 890 ohms. The results are shown in table III and fig. 3.

TABLE III

ELECTRICAL RESISTANCE OF *Laminaria saccharina*

Time in minutes	Resistance	Solution	Resistance of control in sea water
0......	800	Sea water containing 0.099 M ether	780
10......	910		
30......	910		
50......	910		
60......	800	Sea water	....
170......	800		
180......	910	Sea water containing 0.099 M ether	....
200......	910		
220......	910		
240......	910		760
480......	890		

The effect of higher concentrations of ether was next investigated. Tissue which had a net resistance of 760 ohms in sea water was placed in a mixture of 970 cc. sea water+30 cc. ether +15 cc. of concentrated sea water, which was added to make the conductivity of the mixture equal to that of sea water. The concentration of the ether was therefore 2.96 per cent by volume (=0.293 M). In the course of

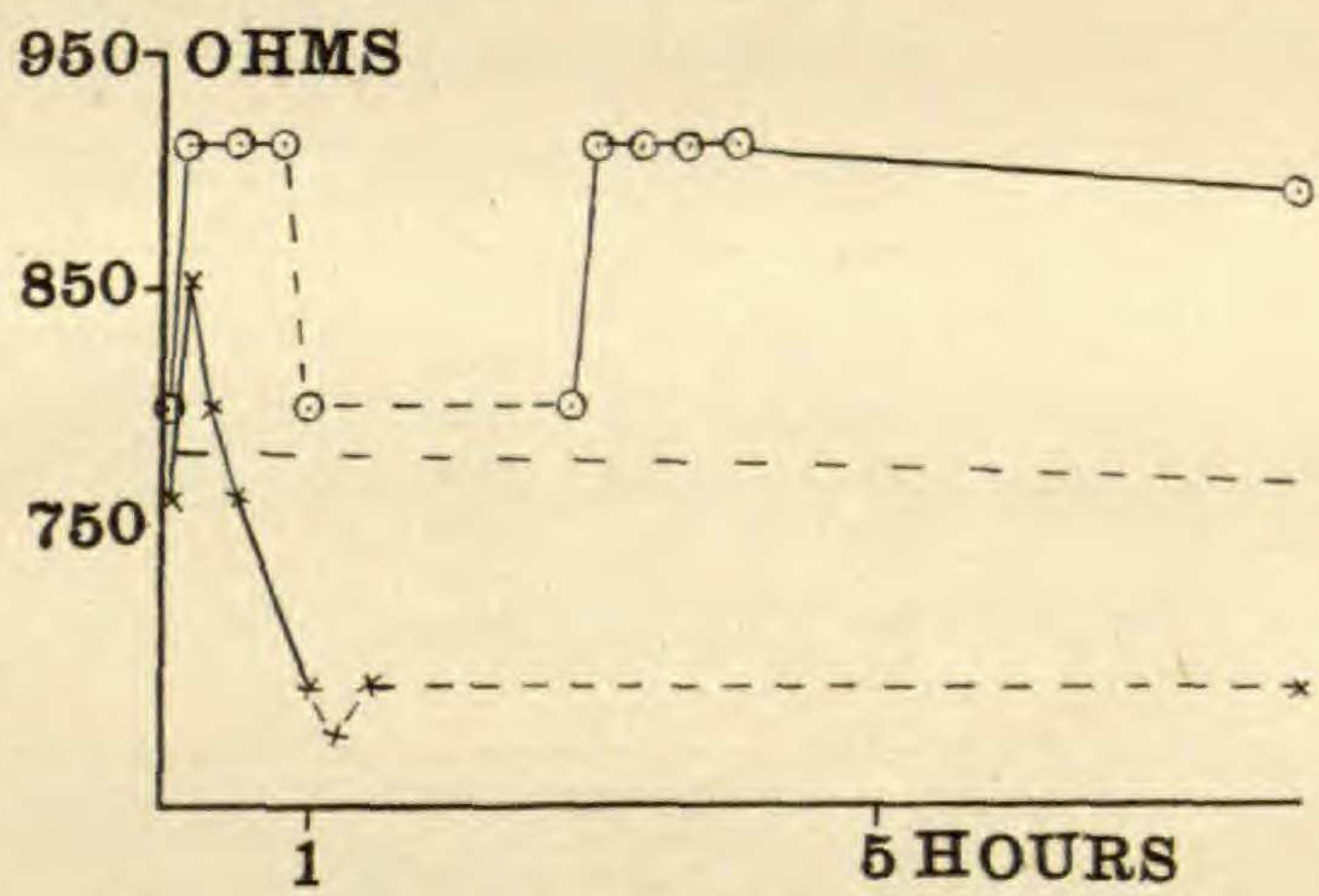

FIG. 3.—The upper curve shows the decrease of permeability (increase of electrical resistance) of *Laminaria saccharina* under the influence of ether (0.099 M in sea water=1 per cent by volume), followed by a rapid return to normal when replaced in sea water (dotted portion of curve), and the rapid decrease of permeability when again placed under the influence of ether: the lowest curve shows the effect of a higher concentration of ether (0.293 M=0.296 per cent by volume); there is a decrease of permeability followed by an increase (fall of electrical resistance) and subsequent slight rise when transferred to sea water (dotted portion of curve); the middle curve (dotted line) shows the electrical resistance of a control in sea water.

10 minutes the resistance rose to 850 ohms; during the next 10 minutes it fell to 800 ohms; it continued to fall rapidly during the next 40 minutes, reaching 680 ohms at the end of this period. The tissue was then placed in sea water; in the next 10 minutes the resistance fell to 660 ohms. This fall in resistance was doubtless due to the continued action of the ether, which required time to diffuse out of the tissue. During the next 10 minutes there was a rise of 20 ohms, which was probably due, either wholly or in part, to the fact that the resistance of the sea water was greater than that of the mixture from which the ether had partly evaporated.[6] During the next 400 minutes no rise occurred. The results are shown in table IV and fig. 3.

TABLE IV

ELECTRICAL RESISTANCE OF *Laminaria saccharina*

Time in minutes	Resistance	Solution	Resistance of control in sea water
0	760	Sea water containing 0.293 M ether	780
10	850		
20	800		
30	760		
60	680		
70	660	Sea water	760
80	680		
480	680		

All readings were taken at 18° C. or corrected to this temperature.

This outcome is very significant, for it shows that the increase of permeability produced by ether is not reversible; while, as we have seen, the decrease of permeability is easily reversed. Since the essential characteristic of an anesthetic is the reversibility of its action, we must associate anesthesia with the reversible decrease of permeability and not with the irreversible increase of permeability.

In view of the importance of this result the experiment was repeated many times, the fall of resistance (before placing in sea water) varying from 50 to 200 ohms, but always with practically the same result. On placing in sea water there were sometimes irregular fluctuations amounting to 20 or 30 ohms but no recovery.

[6] This rise was not always observed, but it might easily have been overlooked when observations were taken only once in 10 minutes.

This result is the more striking inasmuch as material of which the resistance has fallen as much as 150 ohms in NaCl recovers completely when placed in sea water, and may even undergo this treatment daily for several days in succession without injury.[7]

TABLE V

ELECTRICAL RESISTANCE OF *Laminaria saccharina*

Time in minutes	In sea water containing 0.0064 M chloroform (solution renewed every 5 minutes)	In sea water
0	830	850
10	910	....
20	920	....
40	920	....
60	920	850
80	920	....
200	910	....
300	900	850

All readings were made at 18° C. or corrected to this temperature.

The fall of resistance below the normal may be taken as a measure of the toxicity. The toxicity increases with the concentration, and it should be noted that it is greatly decreased if the material is allowed to stand in an open dish, owing to the evaporation of the ether. If the material be placed in a closed jar, oxygen must be supplied. The other alternative, frequent renewal of the solution, is usually preferable.

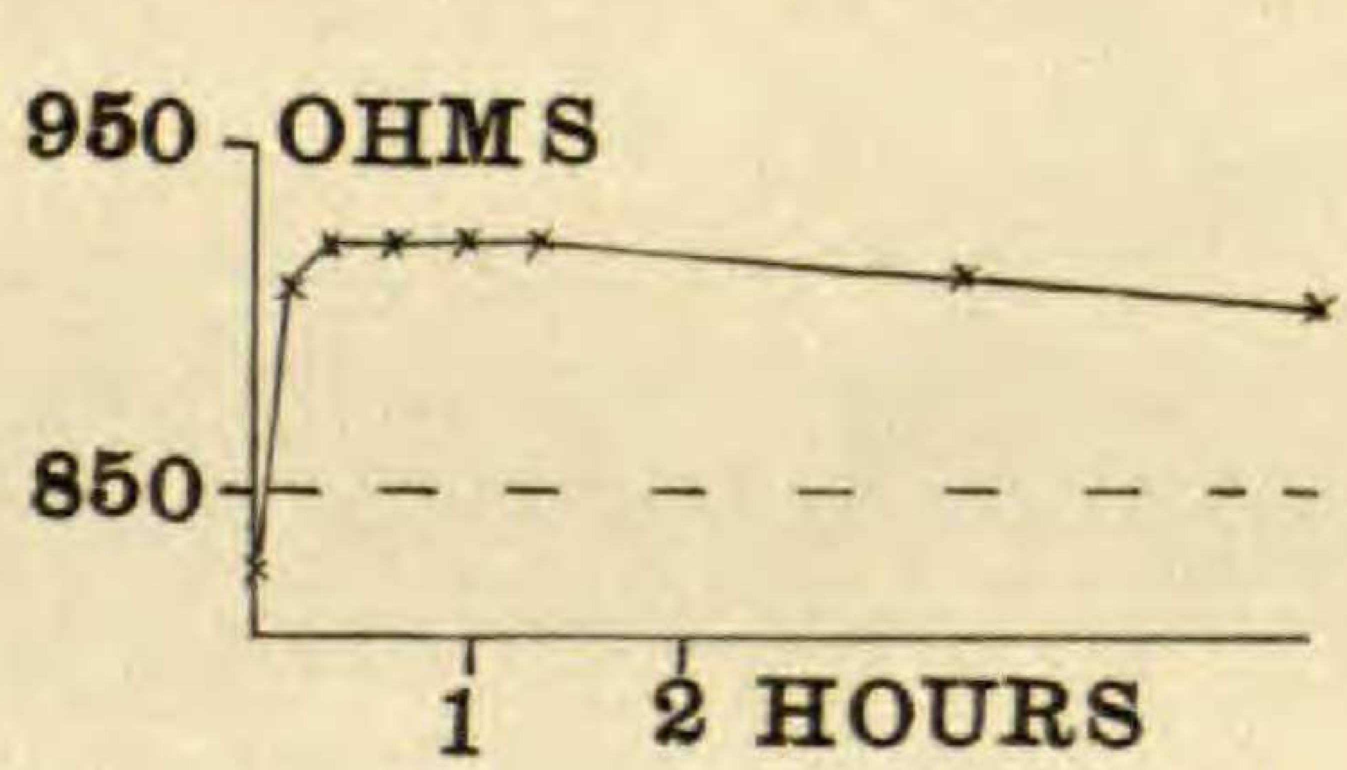

FIG. 4.—Curve showing the decrease in permeability (increase in electrical resistance) of *Laminaria saccharina* under the influence of chloroform (0.0064 M = 0.05 per cent by volume): the dotted line shows the electrical resistance of a control in sea water.

A series of investigations on chloroform gave similar results, the chief difference being that chloroform is much more toxic, and that the concentration necessary for long continued decrease of permeability is much lower, being about 0.05 per cent by volume, or 0.064 M. This is shown by table V and fig. 4, which are the results of an experiment with a mixture containing 999.5 cc. sea

[7] Science N.S. **36**:350. 1912; BOT. GAZ. **59**:242. 1915.

water+0.5 cc. chloroform+0.25 cc. concentrated sea water (this mixture had the same conductivity as sea water). In this

TABLE VI

ELECTRICAL RESISTANCE OF *Laminaria saccharina*

Time in minutes	Resistance	Solution	In sea water
0	760	Sea water containing 0.0128 M chloroform (solution renewed every 5 minutes)	730
10	840		
20	760		
40	720		
70	670	Sea water	
80	660		
100	650		730
120	640		
150	630		
200	620		720
300	610		710

All readings were taken at 18° C. or corrected to this temperature.

experiment the solution was renewed every 5 minutes during the first 80 minutes, and every 15 minutes thereafter. It will be seen that the result is very similar to that obtained with 0.009 M ether.

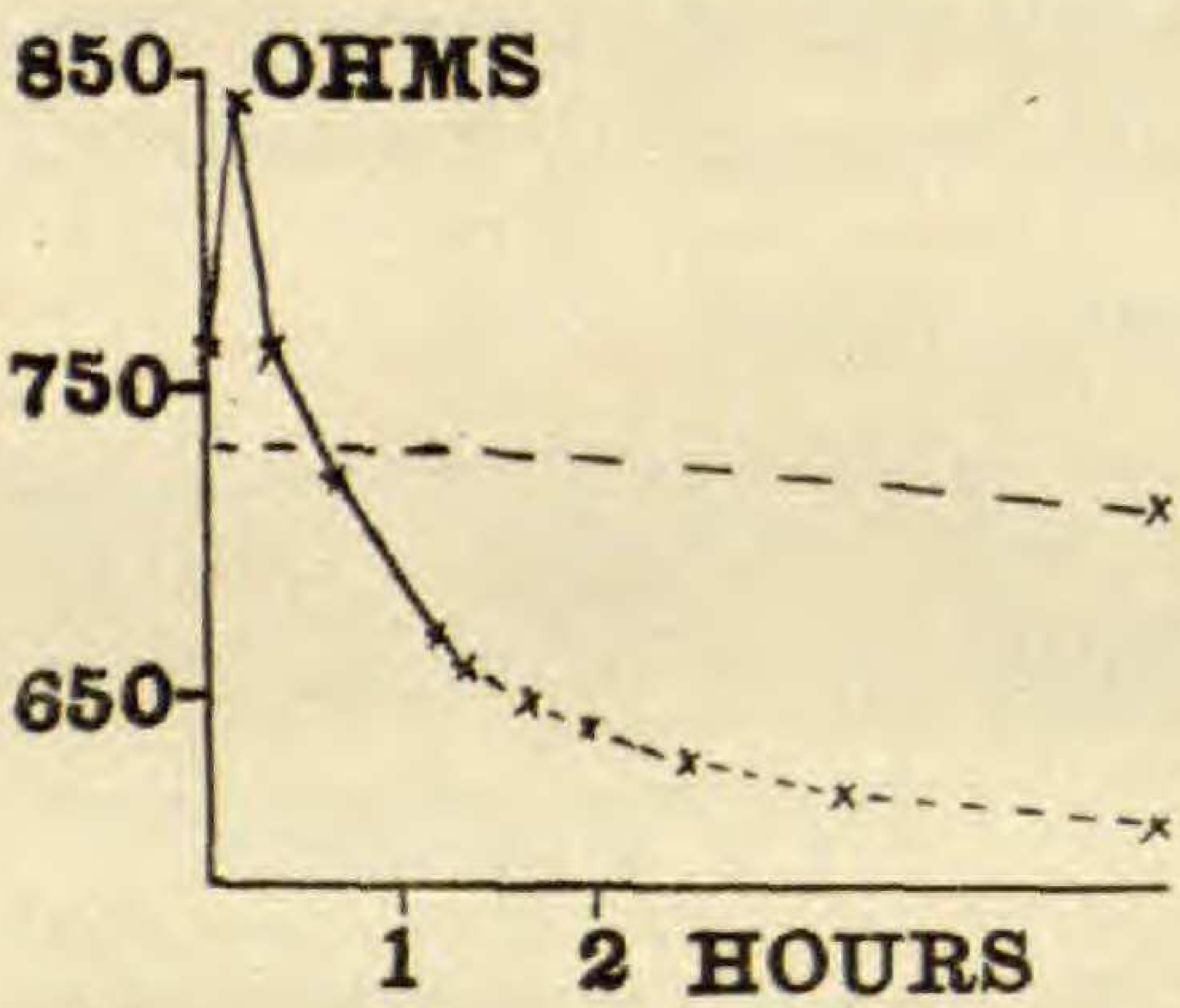

FIG. 5.—Curve showing the rapid decrease followed by an increase of permeability of *Laminaria saccharina* under the influence of chloroform (0.0128 M=0.1 per cent by volume) and the subsequent failure to recover when replaced in sea water (dotted portion of the curve): the dotted line shows the electrical resistance of a control in sea water.

If we increase the concentration of chloroform to 0.1 per cent by volume (=0.0128 M), the result is quite similar to that obtained with 0.293 M ether. This is shown in table VI and fig. 5, which gives the results of an experiment with a mixture containing 999 cc. sea water+1 cc. chloroform+0.5 cc. concentrated sea water (this mixture had the conductivity of sea water). The solution was renewed every 5 minutes during the first 80 minutes, after which it was kept in sea water. There is no indication of recovery after the tissue is replaced in sea water.

Experiments with chloral hydrate gave results very similar to those obtained with chloroform, the corresponding effects being produced in both cases by approximately the same percentage concentrations,[8] that is, chloral hydrate 0.1 per cent (=0.006 M) acts similarly to chloroform 0.1 per cent by volume (=0.0128 M).

The experiments with alcohol lead to somewhat different results. In the first place, alcohol is not as toxic as ether, chloroform, or chloral hydrate, and higher concentrations must be used to produce the same effects on permeability. In sea water containing alcohol 0.051 M or 2.955 per cent by volume (the solution being renewed every 15 minutes) the results were much the same as in 0.099 M ether (the solution being renewed every 5 minutes), except that the rise in resistance took place more slowly, sometimes occupying 30 minutes or more. It was found that 0.2385 M or 13.875 per cent by volume is decidedly toxic.

An interesting feature of the results with alcohol is that the increase of permeability is reversible. If the increase be carried too far it is not reversible (or to a much smaller extent); in the first experiments this condition was unintentionally realized and lead the writer to suppose that alcohol behaves like ether. The course of a typical experiment is shown in table VII and fig. 6. The tissue was first placed in a mixture containing 970 cc. sea water+30cc. Squibb's absolute alcohol+about 15cc. concentrated sea water. This mixture had the conductivity of sea water; the concentration of alcohol was 0.051 M (2.96 per cent by volume). The net resistance rose from 800 to 880 ohms in the course of 40 minutes. The tissue was then placed in sea water containing 0.2385 M alcohol (13.875 per cent by volume), and in the course of 20 minutes the resistance fell to 700 ohms. The tissue was then placed in sea water and the resistance again rose to 800 ohms.

The facts that recovery occurs in alcohol, and that irregular fluctuations are often observed in experiments on recovery from ether, suggest that the difference between the behavior

[8] No effort was made to find the exact percentages which would produce given effects, as this was not the primary object of the investigation. The actual concentration of chloral hydrate may have been somewhat lower than those given, owing to the presence of water in the chloral hydrate.

of alcohol and the other anesthetics investigated is only one of degree. It is probable that there is some recovery in ether,

TABLE VII

ELECTRICAL RESISTANCE OF *Laminaria saccharina*

Time in minutes	Resistance	Solution	In sea water
0	800	Sea water containing 0.051 M alcohol (solution renewed every 10 minutes)	820
20	840		
30	860		
40	880		
50	760	Sea water containing 0.2385 M alcohol (solution renewed every 10 minutes)	820
60	700		
80	800	Sea water	820
200	800		

All readings were taken at 18° C. or corrected to this temperature.

chloroform, and chloral hydrate, but that it is so slight and so transitory as to be difficult of observation.

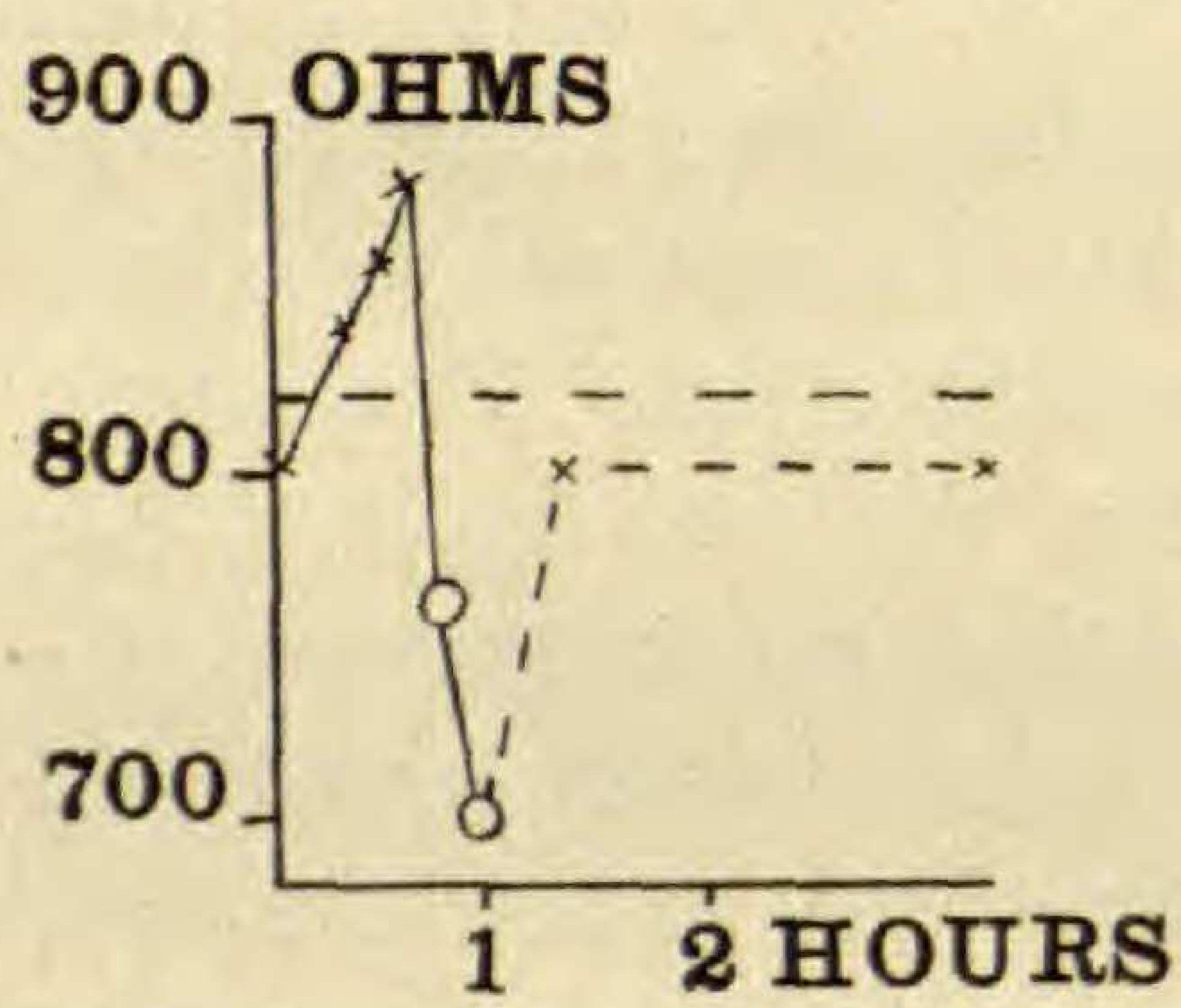

FIG. 6.—Curve showing the decrease of permeability of *Laminaria saccharina* under the influence of alcohol (0.051 M in sea water =0.269 per cent by volume), followed by a rapid increase in permeability when placed in a stronger concentration of alcohol (0.2385 M =13.875 per cent by volume) (portion of curve with circles), followed by recovery when replaced in sea water (dotted portion of curve): the dotted line shows the electrical resistance of a control in sea water.

It is evident that suitable concentrations of anesthetics produce a marked decrease of permeability, which may amount to 15 per cent or even more.[9] This condition may be maintained for a long time if the concentration is not too high; with higher concentrations the period is shortened and may become so short as to be observed with difficulty. This decrease of permeability can be easily and quickly reversed by replacing the tissue in sea water. It does not seem to produce any injury if the concentration be not too high. The relative concentrations necessary to produce this result correspond closely with those required

[9] The amount depends somewhat on the condition of the material. Material in poor condition in general shows less rise in resistance than good material.

to produce anesthesia,[10] being least for chloral hydrate and largest for alcohol.

On the other hand, the increase of permeability (except in the case of alcohol, within certain limits) produces permanent injury and is not reversible. It cannot be regarded, therefore, as the characteristic effect of the anesthetic. The characteristic effect must be regarded as in some way connected with decrease of permeability.

It is easy to see how a decrease of permeability to ions must hinder the production and the transmission of stimuli in so far as these are dependent on the movement of ions in the tissues, and there is abundant evidence that stimulation is always accompanied by such movements of ions in the protoplasm. It seems clear, therefore, that a decrease in permeability may result in the decrease of irritability, which is the characteristic effect of an anesthetic.

These investigations are of interest in view of the fact that MEYER'S theory of anesthesia, which has found wide acceptance, states that anesthesia is the result of an increase of permeability. MEYER supposes that anesthetics act on the lipoids of the cell in such a way that they become more permeable.

On the other hand, LILLIE[11] has developed a theory according to which anesthetics act by rendering the plasma membrane more refractory to changes of permeability either by decreasing its permeability or in some other way. LILLIE has observed that anesthetics antagonize the action of NaCl. This, however, does not by itself tell us anything regarding the action of anesthetics on permeability under normal circumstances, as for example when added to sea water. LEPECSHKIN,[11] on the bases of plasmolytic investigations, states that the entrance of dyes and of KNO_3 into the cell is hindered by anesthetics, but this is disputed by RUHLAND.[11]

Since the announcement of the writer's investigations,[2] similar experiments have been undertaken in HÖBER'S laboratory, with

[10] See, for example, the recent investigations of V. KÖRÖSY, Zeit. Physik. Chemie **93**:145. 1914. He finds that the reversible action of chloroform on cell division in fish embryos and photosynthesis in *Elodea* is confined to practically the same concentrations as those which produce reversible effects in the permeability of *Laminaria* (about 0.0062 M).

[11] Pringsheim's Jahrb. Wiss. Bot. **51**:376. 1912.

the result that they have been completely confirmed.[12] These experiments were made on red blood corpuscles, the permeability being determined by means of electrical measurements.

It is easy to imagine a mechanism which would respond to the action of anesthetics by a decrease of permeability. Since salts are less soluble in ether, alcohol, and chloroform than in water, it is evident that the presence of these substances in the plasma membrane in sufficient concentration would diminish the solubility of salts in the membrane and consequently hinder their penetration. The anesthetics might become more concentrated in the plasma membrane than in the surrounding solution either by chemical combination, by solution, or by absorption. It should be remembered that such salts as Mg, Ca, Al, and La also cause a decrease of permeability, and this suggests that chemical combination or coagulation, rather than mere accumulation of an anesthetic, is responsible for the effect. Moreover, the mere accumulation of an anesthetic at the plasma membrane (without chemical combination or coagulation) could not explain the increase of permeability. The best assumption is that the anesthetic combines chemically[13] with the protoplasm, the effect on permeability changing after a certain amount has combined. Good analogies are offered by cases in which chemical combination with a small amount of substance produces an effect which is reversed as soon as more combines. These analogies seem fairly satisfactory in the present condition of our knowledge.

Laboratory of Plant Physiology
Harvard University

[12] Deutsche medicinische Wochenschrift. no. 10. 1915.

[13] Under the term "chemical combination" coagulation is included.